Remarks

Claims 1-3, 5-8, 10, 11, 15-17, 19 and 20 are pending and under examination. Claim 15 is amended herein with support as noted below. Applicants also note that no new issues are presented by the amendment to claim 15 for the reasons noted below. Thus, applicants believe they are entitled to entry of this amendment, and respectfully request that it be entered and considered at this time.

Preliminary Matters

Regarding the issue of the declarations submitted as Exhibits A-C, applicants understand the Office to be stating that Exhibits A and B have been considered (page 5 of office action). However, in the preliminary matters section, the Office Action states that Exhibits A and C are not of record, and suggests that they be resubmitted. The Office Action also advises applicants that declarations and other evidence submitted after final rejection are not considered timely filed and will not be considered.

Applicants have taken the suggestion to resubmit Exhibits A and C, along with a copy of the date-stamped post card received from the Office, which acknowledges timely receipt of all three Exhibits, A-C. Applicants timely submitted, and the Office timely received, Exhibits A and C along with Exhibit B. The Office Action implies that the resubmitted exhibits will not be considered with the present response. However, the failure of the Office to consider resubmitted Exhibit C would be improper under the present facts. The fact that Exhibit C was lost by the Office after receipt cannot be treated as applicants' failure to timely submit it. The fact that it is being resubmitted after a final Office Action is entirely due to its loss by the Office. The inconvenience of the Office's

error is not properly shifted to the applicant. Thus, the Office should consider applicants' timely filed and currently resubmitted evidence in Exhibit C.

Rejections under 35 U.S.C. § 112

Claims 1-3, 5-8, 10 and 11 are rejected as allegedly lacking enablement for *in vivo* applications. The claims read on both *in vitro* and *in vivo* applications of the recited methods. The Office bases the present rejection on the grounds recited in pages 4-6 of the Office Action. These grounds are individually addressed below.

The *in vivo* effects of the compounds of the claims are known, and are recited in the specification. The specification explicitly provides guidance as to the effects of introducing an acetyltransferase inhibitor into a cell *in vivo*, namely, inhibition of melatonin and increase in serotonin. This is laid out in detail below. Furthermore, the *in vivo* reduction in melatonin taught in the specification is confirmed in the *in vivo* data presented in the previously submitted exhibits, which show administration *in vivo* of a dosage (10mg/kg) taught in the specification in a manner (subcutaneous) taught in the specification (see Exhibit C).

The absence of working examples in the specification is not fatal. Applicants are not legally required to demonstrate the invention. Furthermore, the relevant teachings in the specification of dosage of drug, modes of administration of drug and *in vivo* effects of drug (i.e., how to use) are confirmed in the examples presented in Exhibits B and C, previously presented. Since no steps were practiced in the treatment methods described

in Exhibit C that were not explicitly disclosed in the specification, the fact that the specification is enabling is confirmed. The steps practiced and the successful reduction in melatonin obtained in Exhibit C confirm that the manner of using (how to use) the recited compounds *in vivo* is as taught in the specification.

The assertion that the experiments described in the Declarations used "healthy rats rather than disease models" is incorrect. The isoproteronol-treated rats are abnormal (i.e., diseased) in a highly relevant parameter. There is no assertion in the Office Action that rats with 10-fold elevated melatonin are normal. Thus, it is misleading to suggest that no treatment is taught when the treatment reduced melatonin levels significantly. In fact, the reduction of melatonin production is the explicit object of claim 11, and it is demonstrated. The other method claims are directed to producing bisubstrate inhibitors (claims 1-3 and 5), and inhibiting acetyltransferase activity (claims 6-8 and 10).

Furthermore, it is improper for the Office to assert that the law requires the specification to teach how to use methods consistent with utilities recited in the specification but not claimed. Thus, recitations noted by the Office regarding providing therapeutic benefit, limiting adverse effects of certain drugs and improving the efficacy of certain drugs are not relevant to enablement of the present claims because the present claims do not recited those utilities. In the present case, the only claimed uses are directed to reduction of melatonin production (claim 11), producing bisubstrate inhibitors in cells (claims 1-3 and 5), and inhibiting acetyltransferase activity (claims 6-8 and 10). It is these effects only that require enablement *in vivo*, and they do not require demonstration, only a scientifically supportable basis to believe they will work. The data

date in the application provides this. Nevertheless, the successful practice of claim 11 *in vivo* demonstrated in the declarations establishes that the methods of claims 1-3, 5, 6-8 and 10 are also enabled *in vivo*, because the effects recited in these method claims underlie the *in vivo* effect recited in claim 11 and demonstrated *in vivo* in the declaration. Thus, the data in the declarations confirm that the detailed teachings in the specification are enabling *in vivo* for the effects claimed.

Applicants further point out that there is no basis asserted by the Office why the *in vivo* effects are not accepted to be the same as the *in vitro* effects. For scientific and logical reasons, this should be the starting point of this inquiry. The mechanism of action is a chemical reaction that is shown to happen in cells. In the absence of some stated reason to believe that this intracellular effect would be different depending on where the cell is, this ground for rejection is unsupported. In fact, applicants have verified that the *in vivo* effects are the same for melatonin production. Since the other effects recited in the claims are necessary precursors to the demonstrated in vivo effect on melatonin production, it must be assumed that they are occurring in vivo.

The specification itself teaches how to use the invention, by providing specific guidance for the *in vivo* method steps themselves (e.g., administration modes and doses), for determining the presence of the recited effect *in vivo* (e.g., production of a bisubstrate inhibitor, inhibition of an acetyltransferase, reduction in melatonin or increase in serotonin) and for assessing toxicity. Examples of the teaching of the specification on these points are detailed below.

The specification enables the determination of the effect of the recited method steps in accomplishing the specified (claimed) effect, i.e., inhibition of melatonin synthesis or increase in the amount of serotonin. For example:

"That melatonin production has been inhibited in the cell by the method of this invention can be determined according to assays well known in the art for measuring melatonin production" (page 7, lines 7-9);

"In an intact subject, melatonin production can be monitored by measurement of the major melatonin metabolite, 6-sulfatoxymelatonin" (page 9, lines 2-4); and

"That the amount of serotonin in the cell has been increased by the method of this invention can be determined according to assays well known in the art for measuring an amount of serotonin" (page 7, lines 21 -24).

The specification enables the determination of the effect of the recited method steps in accomplishing the specified effect, i.e., production of a bisubstrate inhibitor and inhibition of an acetyltransferase:

"***AANAT assay:*** The specific activity of the AANAT preparation was determined from measurement of the activity of the enzyme and amount of protein in a preparation. Activity was assayed by incubating the enzyme with ^{3}H-acetyl CoA (0.5 mM, 4μCi/μmole) and tryptamine hydrochloride (1 mM) , bovine serum albumin (0.5 mg/ml) and the enzyme in total volume of 100 μl of sodium phosphate (0.1M, pH 6.8) (6,7). The incubation was terminated by extracting the product ^{3}H-acetyltryptamine with chloroform (1 ml). The chloroform phase was washed sequentially with 200 μl of the sodium phosphate buffer and twice with 200 μl of NaOH (1N). The radioactivity in 400 μl of the chloroform phase was determined following evaporation of chloroform under vacuum. Protein was measured by optical density at 280 nm and by dye binding using the Brandford procedure."
(page 18, lines 9-19)

In the context of the present methods, it is recognized that demonstrated inhibition of an acetyltransferase (e.g., AANAT) is an indicator of the production of a bisubstrate

inhibitor. The methods known in the art include methods known to be applicable to determining these effects *in vivo* as well as *in vitro*.

Furthermore, the application teaches monitoring of the intended specific effect on melatonin (e.g., physiological and psychological) and monitoring of general indices of metabolism as indicators of nonspecific effects in a subject, which includes an assessment of toxicity:

"For example, a substrate derivative of this invention can be administered to a subject and the intended specific effect of the drug in the subject can be monitored, along with general indices of metabolism as an indication of nonspecific effects. From these results, compounds can be identified which have the strongest intended effect relative to non-specific effects. As a specific example, in the case of arylalkylamine N-acetyltransferase, melatonin production by pineal cells can be monitored as an index of a specific effect at the cellular level and protein synthesis, RNA synthesis and cell viability can be monitored as indices of non-specific effects at the cellular level. In an intact subject, melatonin production can be monitored by measurement of the major melatonin metabolite, 6-sulfatoxymelatonin and nonspecific effects can be monitored in the subject by measuring such parameters as water intake, food intake, weight gain, locomotor activity and body temperature. More sophisticated tests can include various psychological indices, such as problem solving, memory, and aggressiveness."
(page 8, line 26 to page 9, line 7)

"In the methods of the present invention which describe the treatment of a disorder by administering a substrate derivative of this invention to a subject, the efficacy of the treatment can be monitored according to clinical protocols well known in the art for monitoring the treatment of the particular disorder."
(page 11, lines 15-18))

The application teaches the manner of using the compounds *in vivo*, i.e., how to administer the substrate derivatives of the invention to a subject:

"The substrate derivatives employed in the methods of this invention can be administered to a cell either *in vivo* or ex vivo. Thus the substrate derivatives of the present invention can be in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may

be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects in a subject or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art."
(page 10, lines 5-14).

Specific guidance on modes of administration of the compounds and guidance for determining dosages for *in vivo* uses of the invention are taught in the specification:

"For *in vivo* administration, the substrate derivatives can be administered to a subject orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, intranasally, topically or the like. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the substrate derivative required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disorder being treated, the particular substrate derivative used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every substrate derivative. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein and what is available in the art (21)[Martin, E.W. (ed.) Remington's Pharmaceutical Sciences, latest edition. Mack Publishing Co., Easton, PA.]."
(page 10, line 26 to page 11, line 5)

"Parenteral administration of the substrate derivative of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein."
(page 11, lines 7-13)

Specific examples of *in vivo* dosing and evaluation of the *in vivo* effect of those dosages is provided in the specification for human and non-human animals:

"For example, a human subject (patient) diagnosed with a depression is treated by oral administration of an alkylating derivative of an acetyl acceptor substrate of serotonin N-acetyltransferase (AANAT), (e.g., bromoacetyl tryptamine or chloroacetyl tryptamine) in

a dosage range from about 1 to about 10 mg per kg of body weight, 1 to 4 times a day. The patient is monitored for general physical signs to evaluate nonspecific effects of treatment and by analysis of blood chemistry to identify changes in salt balance and liver function. Efficacy of the treatment is evaluated using standard indices of depression well known in the art of psychiatry."
(page 11, lines 20-27)

"A non-human subject is administered a substrate derivative of this invention orally and/or by subcutaneous injection of the substrate derivative in solution or as a suspension, in a dosage range from about 1 to about 10 mg per kg body weight. The subject is monitored by evaluation of activity cycles, food intake, water intake, general behavior, posture and other such parameters as are well known in the art for evaluation of non-human subjects."
(page 11, line 29 to page 12, line 3)

The specification provides extensive and explicit guidance and examples as to the specific inhibitors that can be used with the recited acetyltransferase (AANAT) in the present claims:

"An example of an important acetyltransferase is provided by serotonin *N*-acetyltransferase (arylalkylamine N-acetyltransferase, AANAT, E.C. 2.3.1.87), which binds AcCoA and a narrow set of arylalkylamines including serotonin, tryptamine, and phenylethylamine, and releases CoA and the corresponding N-acetyl-arylalkylamine, i.e. N-acetylserotonin, N-acetyltryptamine and N-acetylphenylethylamine (1,2) . This enzyme is of biological importance because it is involved in a broad range of biological processes through the key role it plays in regulating the synthesis of melatonin (N-acetyl 5-methoxytryptamine)(3)."
(page 1, line 27 to page, line 3)

"As an example, the acetyltransferase of this invention can be, but is not limited to, any of the acetyltransferases listed in Table 1 and the alkylating derivative of the acetyl acceptor substrate (derivative substrate) of this invention can be, but is not limited to, any of the N-bromoacetylated substrate derivatives in Table 1, listed opposite the respective acetyltransferase upon which it can act in an inhibitory manner. However, it is to be understood that the bromoacetyl group of any of the substrate derivatives in Table 1 can be substituted for a chloroacetyl group or a fluoroacetyl group to produce a substrate derivative of this invention."
(page 6, lines 6-13)

"As a more specific example, when the acetyltransferase of the present invention is serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase (AANAT, E.C. 2.3.1.87)), which regulates the synthesis of melatonin, the alkylating derivative of the

acetyl acceptor substrate can be, but is not limited to, N-bromoacetyltryptamine, N–bromoacetylserotonin, N-bromoacetylphenylethylamine, N-bromo-acetyl-methoxytryptamine, N-bromoacetyltyramine, N-chloroacetyltryptamine, N–chloroacetylserotonin, N-chloroacetylphenylethylamine, N-chloro-acetyl-methoxytryptamine, N-chloroacetyltyramine, N-fluoroacetyltryptamine, N–fluoroacetylserotonin, N-fluoroacetylphenylethylamine, N–fluoro-acetyl-methoxytryptamine and/or N-fluoroacetyltyramine, as well as any other alkylating derivative of an acetyl acceptor substrate for AANAT that is now known or later identified."
(page 6, lines 15-26)

For the claimed method of inhibiting melatonin production, the specification explicitly teaches the specificity of the inhibitors to be used:

"Thus, the present invention further provides a method of inhibiting melatonin production in a cell which produces melatonin, comprising introducing into the cell an alkylating derivative of the acetyl acceptor substrate of AANAT which can be, but is not limited to, N-bromoacetyltryptamine, N-bromoacetylserotonin, N–bromoacetylphenylethylamine, N-bromo-acetyl-methoxytryptamine, N–bromoacetyltyramine, N-chloroacetyltryptamine, N-chloroacetylserotonin, N–chloroacetylphenylethylamine, N-chloro-acetyl-methoxytryptamine, N–chloroacetyltyramine, N-fluoroacetyltryptamine, N-fluoroacetylserotonin, N–fluoroacetylphenylethylamine, N-fluoro-acetyl-methoxytryptamine and/or N-fluoroacetyltyramine, as well as any other alkylating derivative of an acetyl acceptor substrate for AANAT that is now known or later identified."
(page 6, line 28 to page 7, line 9)

In summary, there is extensive guidance in the specification regarding the *in vivo* effects of the class of compounds disclosed, the manner of using the compounds *in vivo* to achieve the claimed effects, the guidance as to the required specificity of the inhibitor, and assessment of toxicity. These extensive teachings are validated by the results of the two *in vivo* treatment methods described in the Exhibits submitted with the previous response and resubmitted (Exhibit C) herewith.

The Office does not provide any specific scientific basis to challenge the veracity of the substantial statements made in the application regarding *in vivo* enablement of the claimed methods. In order for the Office to not take what applicants' state at face value, a reasonable basis must be presented as to why applicants' statements should not be believed. In re Wright, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure). A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. MPEP Section 2146.04. On its face, applicants' specification uses "terms which correspond in scope to those used to define the subject matter sought to be patented": the specification describes methods to make an inhibitor in a cell in a subject and that our method can decrease melatonin production in a subject, and this is what we claim. This teaching is exemplified above. Thus, where the application makes an affirmative statement on every point raised by the Office, the Office must do more than state that applicants don't teach or provide guidance on that point.

If the Office believes that applicants statements are inaccurate or insufficient "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to

back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). Any such evidence is missing in the present Office Action. The Office does not give any evidence to contradict applicants' teaching that the methods will work *in vivo*, or to assert that there is a flaw in the recited underlying science or that the specific guidance provided is inaccurate. The Office relies only on its repeated assertion that guidance is lacking to support its conclusion that applicants don't meet the how to use requirement of section 112. There is no supporting evidence or reasoning presented by the Office to support this conclusion. This is insufficient to overcome the weight of the substantial guidance and affirmative statements of efficacy provided in the application. It also seems to ignore the highly relevant proof of efficacy provided by the declarations of Dr. Klein. The detailed teaching of the application of how to practice the methods in humans, taken with validation of efficacy, should control the present analysis, and lead to a conclusion of enablement. Thus, withdrawal of this rejection of claims 1-3, 5-8, 10 and 11 is respectfully requested.

Claims 15-17, 19 and 20 are rejected as allegedly lacking enablement. One ground of the rejection is based on the issue of *in vivo* enablement, and asserts that the cell is not enabled *in vivo*. As another basis for rejecting these claims, the Office states that the bisubstrate inhibitor does not actually comprise the bromoacetyl group or the chloroacetyl group, since the bromo- and chloro- substituents are leaving groups in the enzymatic reaction.

Regarding the *in vivo* aspect of the rejection, the Office's rationale is stated to be the same as for the rejection of the method claims on *in vivo* enablement. Likewise, applicants' remarks in response to those rejections are applicable to the rejection of these claims. On this basis, withdrawal of the rejection is respectfully requested.

Regarding the lack of clarity in claim 15, due to the apparent reference in the claim to N-bromoacetylated acetyl acceptor substrate and N-chloroacetylated acetyl acceptor substrate as a bisubstrate inhibitor, applicants herein amend claim 15. As originally presented, claim 15 recited: "the bisubstrate inhibitor comprises an alkylating derivative of an acetyl acceptor substrate..." In the previously presented version applicants deleted "an alkylating derivative of an" and inserted "a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated" In claim 15 as currently amended, the deleted language is reinserted such that the claim now makes clear that the bisubstrate inhibitor comprises an alkylating derivative of a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated acetyl acceptor substrate. This is believed to address the lack of clarity introduced in the previous amendment. The present amendment is supported in claim 15 as filed and throughout the specification where the relationship between the bisubstrate inhibitor and the N-bromo- and N-chloro-acetylated acetyl acceptor substrates is described. This amendment presents no new issues since it is essentially the re-insertion of language that was previously considered in a previous version of this same claim. Thus, none of the words themselves, their order, or their meaning in the context of the claim requires a new search or a determination of support.

Thus, no new issue is presented that could justify the refusal to enter the present amendment and response.

Credit Card Payment Form PTO-2038 authorizing payment in the amount of $120.00 (large entity fee for one-month extension of time) is enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required or to credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

Gwendolyn D. Spratt
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CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8

I hereby certify that this correspondence, including any items indicated as attached or included, is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date indicated below.

Gwendolyn D. Spratt — Date: 3-29-05

EXHIBIT A

DOCKET NUMBER 14014.0342
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Klein et. al.	)	
	)	Group Art Unit: 1632
Serial No. 09/374,742	)	
	)	Examiner: Baker, A.
Filed: August 13, 1999	)	
	)	
For: "METHODS AND COMPOSITIONS FOR BISUBSTRATE INHIBITORS OF ACETYLTRANSFERASES"	)	

DECLARATION OF DR. DAVID KLEIN UNDER 37 C.F.R. § 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200
The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

I, David Klein, a citizen of the United States of America, residing at 6112 Tilden Lane, Rockville, MD 20852, declare that:

1. I am a co-inventor of the above-referenced patent application and of the subject matter described and claimed therein.

2. I have a Ph.D. degree in biology from Rice University in Houston, Texas. I have been conducting research in the field of endocrinology since 1965 and am a co-author of at least 300 publications relating to endocrinology in general, the majority of which deal with arylalkylamine N-acetyltransferase and melatonin production. I am currently Chief of the Section on Neuroendocrinology, Laboratory of Developmental Biology at the National Institute of Child Health of the National Institutes of Health in Bethesda, Maryland.

3. Data presented in the above referenced application show that halogenated acetyltryptamines can, in fact, act as substrates for acetyl transferases in general and AANAT in particular. Furthermore, the activation group can be any halogen. In particular, data presented below show that, for example, both bromoacetyl activated acetyltryptamines and chloroacetyl activated acetyltryptamines form CoA conjugates intracellularly.

4. Specifically, data generated in my laboratory demonstrate that both N-bromoacetyltryptamine (BAT) and N-chloroacetyltryptamine (CAT) inhibit melatonin production by intact pinealocytes. These data were generated from experiments conducted according to the following protocol.

Pinealocytes were prepared as described in the present application. On the first day, they were treated for 6 hours (1600-2200 hours) with either: a) DMSO, b) DMSO+norepinephrine (NE) (10μM), c) NE+CAT (0.5μM), or d) NE+BAT (0.5μM). CAT and BAT were dissolved in DMSO. The cells were then washed twice and incubated in normal medium overnight. The cells were treated on day two (1000-1500) as described below (N=3). Results of these studies are shown in Table 1A below.

5. Table 1A.

Treatments on Day One (6hr)	Treatments on Day Two (5hr)	MT (pmole/10^5 cells)	NAT (μmol/h/10^5 cells)
DMSO	Control	1.07 ± 0.09	Not detectable
	NE 10 μM	10.13 ± 3.96	1.36 ± 0.33 *
	+ BAT (0.5 μM)	1.18 ± 0.41	0.30 ± 0.13
	+ CAT (0.5 μM)	1.21 ± 0.53	0.48 ± 0.08
NE (10 μM, 6hr)	Control	1.27 ± 0.13	ND
	NE 10 μM	9.46 ± 0.22	0.92 ± 0.13
NE + BAT (0.5 μM) 6hr	Control	1.24 ± 0.12	ND
	NE 10 μM	8.05 ± 0.52	0.76 ± 0.14
NE + CAT (0.5μM) 6hr	Control	1.98 ± 0.41	ND
	NE 10 μM	10.45 ± 0.91	0.84 ± 0.19

6. It was observed that, although melatonin production was inhibited, the cells were not killed. This is indicated by the fact that after treatment on day one with each chemical, the cells responded normally on the next day. It is also noted that AANAT activity did not appear to be inhibited. This is because when the cells were assayed for activity, they were homogenized and dissolved in a large volume of buffer. This diluted BAT, CAT and derivatives, so that they were no longer present at concentrations sufficient to inhibit.

7. These data demonstrate that both bromoacetyl activated tryptamines and chloroacetyl activated tryptamines inhibit the activity of AANAT in the cell, as indicated by inhibition of melatonin production. The mechanism of action is likely to be via formation of CoA-S-acetyltryptamine, as catalyzed by AANAT.

8. It is well known in the art that the bromoacetyl group (or chloroacetyl group) is an acylating group that will react with a variety of compounds. The synthetic incorporation of this group into other compounds will confer acylating activity on the resulting product. For example, incorporation of the bromoacetyl group into tryptamine results in bromoacetyltryptamine, which is reactive; bromoacetyltryptamine will react chemically with CoA-SH to form CoA-S-N-acetyltryptamine.

CoASH exists in the cell and is generated when one of many acylCoA compounds donate the acyl group. The most common acylCoA is acetyl CoA, a common acetyl donor group. It participates in many acetyltransferase reactions catalyzed by highly specific acetyltransferases which transfer acetyl moieties from AcCoA to a number of different acetyl molecules, such as tryptamine, through a common mechanism. These enzymes are believed to act by binding both AcCoA and the acetyl group donor at the same time and optimally positioning them to favor acetyl transfer. One such acetyltransferase is arylalkylamine –acetyltransferase (AANAT).

AANAT binds AcCoA and tryptamine and generates CoASH and acetyltryptamine. It can also bind acetyltryptamine and CoASH, but it is very improbable that is will generate acetylCoA and tryptamine from these reactants, based on energy considerations. AANAT binds bromoacetyltryptamine and SHCoA and favors the formation of CoA-S-N-acetyltryptamine because it positions them in an ideal relationship which favors acylation of the SH group of CoASH.

Thus, it would be recognized by one skilled in the art that any other acetyltransferase that functions like AANAT by positioning AcCoA and the specific substrate will also bind CoASH and the bromoacetyl-derivatized form of the substrate of that enzyme and catalyze the formation of the CoA-S-N-acetylated substrate and that this will act as a highly specific bisubstrate inhibitor. Thus, the halogen activation group can be used in a large number of different acetyltransferase systems which proceed by similar mechanisms, but have different substrate recognition parameters (See Table 1 of the specification for an exemplary listing of such systems to which this invention applies).

9. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

DAVID KLEIN

DATE

EXHIBIT C

DOCKET NUMBER 14014.0342U2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

re Application of

lein et. al.

erial No. 09/374,742

led: August 13, 1999

or: "METHODS AND COMPOSITIONS FOR BISUBSTRATE INHIBITORS OF ACETYLTRANSFERASES"

Group Art Unit: 1632

Examiner: Anne Marie Falk

DECLARATION OF DR. DAVID KLEIN UNDER 37 C.F.R. § 1.132

ssistant Commissioner of Patents
'ashington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
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The Candler Building
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Atlanta, Georgia 30303-1811

1. I, David Klein, a citizen of the United States of America, residing at 6112 Tilden ine, Rockville, MD 20852, declare that:

2. 1. I am a co-inventor of the above-referenced patent application and of the subject atter described and claimed therein.

3. 2. I have a Ph.D. degree in biology from Rice University in Houston, Texas. I have en conducting research in the field of endocrinology since 1965 and am a co-author of at least 0 publications relating to endocrinology in general, the majority of which deal with ylalkylamine N-acetyltransferase and melatonin production. I am currently Chief of the ction on Neuroendocrinology, Laboratory of Developmental Biology at the National Institute Child Health of the National Institutes of Health in Bethesda, Maryland.

4. The *in vivo* effect of 10 mgs/kg BAT on intracellular AANAT activity was vestigated using rats. Intracellular AANAT activity was estimated by measuring the amount of elatonin in the pineal gland because melatonin levels are regulated by the intracellular activity AANAT. Animals were injected with isoproterenol, which is known to elevate AANAT otein and the broken cell activity of the enzyme. In addition, some animals received BAT. his treatment suppressed intracellular AANAT activity, as indicated by reduced levels of elatonin in the pineal gland.

5. Intracellular AANAT activity was estimated by measuring the amount melatonin in e pineal gland, which is known to be closely correlated with the rate of melatonin production, hich in turn is controlled by AANAT activity. AANAT protein was increased by treating rats ith an adrenergic agent, isoproterenol. Some animals were also treated with BAT to determine this depressed the amount of melatonin in the pineal gland.

6. The animals used in the study were 100 gram Sprague Dawley male rats. Drugs were ministered subcutaneously(sc). At 10:00 to 10:20 AM groups of animals were injected with oproterenol (20 mg/kg) or saline. At 11:30 to 11:50 AM one of the isoproterenol-treated oups and one saline treated group received BAT (10 mg/kg). At 12:00 to 12:20 animals were lled and the pineal glands were removed. The treatment and sacrifice times were adjusted so at animals were treated with isoproterenol for 120 minutes and with BAT for 30 minutes prior removal of the pineal gland. Melatonin was measured by radioimmunoassay.

7. Isoproterenol(20 mg/kg sc) treatment increased melatonin content of the pineal gland ~ 10-fold. BAT treatment did not reduce pineal melatonin in control rats, but did reduce this ~ 25% of the mean value in isoproterenol-treated rats from 42.97 ± 25.63 to 12.43 ± 3.19 oles of melatonin per gland (Table 1).

8. Table 1 Effect of bromoacetyltryptamine (BAT, 10 mg/kg) on melatonin in e pineal gland of control and isoproterenol-treated (20 mg/kg) rats. The numbers in parenthesis dicates the number of animals in a group.

Group	Treatment	Pineal melatonin (pmols/gland)
1	Control	3.5 ± 1.24(6)
2	Control + BAT	6.95 ± 1.43(6)
3	Isoproterenol	42.97 ± 25.63 (7)
4.	Isoproterenol + BAT	12.43 ± 3.19(6)*

*P> 0.01 as compared to group 3 (Mann Whitney two-sample rank *U* test).

9. This study demonstrates that BAT can act *in vivo* to inhibit the activity of AANAT nd provides evidence that it or a related compound may have utility as a drug that regulates ANAT activity in humans.

10. I further declare that all statements made herein of my own knowledge are true, and nat all statements made on information and belief are believed to be true; and further, that these tatements were made with the knowledge that willful false statements and the like are unishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code nd that such willful false statements may jeopardize the validity of the application or document r any patent issuing therefrom.

DAVID KLEIN

DATE 31 DEC 200

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COMMISSIONER FOR PATENTS
SIR: PLEASE ACKNOWLEDGE RECEIPT OF THE ENCLOSED:

1. Amendment w/Appendix 1 (24 pages)
2. Exhibits A, B and C (17 pages)
3. Certificate of Mailing: January 2, 2003

IN RE APPLICATION OF: Klein et al.

TITLE: "METHODS AND COMPOSITIONS FOR BISUBSTRATE INHIBITORS OF ACETYLTRANSFERASES"

SERIAL NO.: 09/910,588 FILED: July 20, 2001

REF. NO.: 14014.0342U2 (GDS/DEH:kjb)

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Date 1/14/03